

2. RESPONSE/REMARKS

2.1 STATUS OF THE CLAIMS

Claims 1-46, and 49-66 were pending at the time of the Action and examined on the merits.

Claims 18, 34-46, 49-55, 57-58, and 60-61 have been withdrawn.

Claims 1, 10, 16, 17, 19, 24-27, 56, 59, 62-66 have been amended herein.

Claims 20-23, 28-33 have been canceled herein without prejudice and without disclaimer.

Claims 1-19, 24-27, 34-46 and 49-66 remain pending in the case.

2.2 AN INFORMATION DISCLOSURE STATEMENT IS PROVIDED.

The Action at page 4 objected to the Information Disclosure Statement as allegedly not complying with the requirements of 37 C. F. R. § 1.98(a)(2).

Applicant respectfully traverses. However, in the interest of proceeding the case to allowance without further delay, a new Information Disclosure Statement is provided herewith that lists the references of record, and provides copies of each of the cited references. Applicant submits this rejection is now overcome, and respectfully requests its withdrawal.

2.3 THE OBJECTION TO CLAIMS 10, 17, 18, 25-27, 31 AND 32 IS OVERCOME.

The Action at pages 5-6 objected to claims 10, 17, 18, 25-27, 31, and 32 as allegedly containing informalities.

Applicant appreciates the helpful suggestions of the Examiner in correcting these inadvertent typographical errors. Applicant also notes that claims 18, 31, and 32 have been

withdrawn making their objection moot. Applicant now submits that these objections are overcome, and respectfully requests they be withdrawn.

2.4 REJECTION OF CLAIMS UNDER 35 U. S. C. §101 IS RENDERED MOOT.

The Action at page 6 rejected claims 20, 22, 23, 28, 29, AND 33 under 35 U. S. C. § 101, allegedly because the claimed invention is directed to non-statutory subject matter.

Applicant respectfully traverses; however, since these claims have been canceled, the rejection is moot. Applicant respectfully requests that the rejection be withdrawn.

2.5 REJECTION OF CLAIMS UNDER 35 U. S. C. §112, 1ST PARAGRAPH, IS OVERCOME.

The Action at page 6-12 rejects claims 1-33, 56, 59-66 under 35 U. S. C. § 112, 1st paragraph, allegedly as failing to comply with the enablement requirement.

Applicant respectfully traverses. However, in the interest of advancing certain claims of commercial importance to early allowance, Applicant has made the following amendments. Applicant notes that such amendments are in no way an indication of Applicant's acquiescence to the Examiner's rejection, and expressly reserves the right to re-file claims directed to the original scope of the amended claims.

Claims 1 and 63-65 have been amended by restricting the enucleated recipient cell to either an enucleated oocyte or an enucleated stem cell. These claims have been further restricted by including a proviso excluding the possibility of producing a primate embryo.

Claim 16 has been amended by restricting to the production of a **non-primate** embryo and to the use of an enucleated recipient **oocyte**. Additional steps such as embryo activation,

culturing to a suitable stage of development and transferring to a female surrogate have also been inserted for clarity.

Claims 19, 24 and 59 have been amended by restricting to a **non-primate** embryo or animal.

Claims 25 and 62 have been amended by deleting reference to humans from the claim scope.

Claim 56 has been amended by inserting an additional step of transferring the embryo to a surrogate female, for clarity.

Claim 66 has been amended by inserting a proviso excluding the possibility of producing a primate embryo.

The Applicant contends that the claims as amended are fully enabled by the Specification, which teaches methods of nuclear transfer to produce non-primate embryos using suitable donor and recipient cells. While not restricting the donor or recipient cell type to a non-primate donor or recipient cell, the claims have been amended to include a proviso excluding the possibility of producing a non-primate embryo. The recipient cell has also been amended to recite an oocyte or a stem cell.

As the examiner is aware, nuclear transfer simply involves the removal of the nuclear DNA from one cell (termed the “recipient”) which is replaced with the nuclear DNA of another cell (termed the “donor”). There is sufficient evidence from the literature to support the use of both oocyte and stem cell cytoplasm for use in nuclear transfer to generate either cloned animals or reprogrammed cell fate, respectively.

If the recipient is an oocyte, and the donor a somatic cell, then there is the potential to create a cloned embryo, after activation and embryo culture, and which if transferred to the

reproductive tract may result in a cloned fetus and offspring. This is reproductive cloning and the source of oocytes, activation and embryo culture methods to enable this are known by those skilled in the art.

However, if the recipient cell is from an existing embryonic stem (ES) cell line, and the donor is from a somatic cell, then the nuclear DNA in the somatic cell may be dedifferentiated (reprogrammed) in the resulting hybrid cell after nuclear transfer, under the influence of the embryonic stem cell cytoplasm. This process *does* involve nuclear transfer, but does not yield cells that are capable of forming an embryo or offspring on their own. Thus, these hybrid NT cells are not totipotent in their own right. Yet the hybrid cells may regain sufficient developmental plasticity, so that given the appropriate environmental cues, they may be directed along specific developmental paths to have utility in regenerative medicine if performed with human ES and somatic cells as recipients and donors, respectively.

Previous literature from 1997 demonstrates that pluripotent stem cell cytoplasm can be used to reprogram somatic cells, in the mouse *Tada et al.*, 1997. These hybrid cells contained both sets of nuclear DNA; one from the stem cell and the other from the somatic cell. In essence, this study confirms the origin of other forms of cytoplasm that could be used successfully in nuclear transfer. Thus, it is reasonable to expect that by taking a selected somatic donor cell in G1 of the cell cycle and fusing that to a stem cell cytoplasm would result in a proportion of successfully reprogrammed cells. Since the filing of the present application, this prediction has been substantiated further with experiments in mouse and humans, with potential benefit for regenerative medicine (*Tada et al.*, 2003; *Cowan et al.*, 2005).

Regarding methods that are directed to the cloning of a non-primate mammal, the recipient cell has been restricted to an enucleated oocyte. In addition, and as mentioned above,

the relevant claims have been amended by referring to the step of transferring an embryo into a surrogate of the same or a *closely related* species. A person skilled in the art would know that in order to produce a cloned mammal, the embryo would need to be gestated in a suitable uterine environment using a recipient (or surrogate) female from the same or closely related species, compatible with development. here are examples were recipient females can be used successfully from a closely related species. A few cases are provided below:

1. Cloned embryos produced using *Ovis orientalis musimon* donor nuclei transferred into domesticated sheep, *Ovis aries* (*Loi et al.*, 2001)
2. Cloned embryos produced using *Bos gaurus* donor nuclei transferred into *Bos taurus* domesticated cattle (*Lanza et al.*, 2000; *Vogel*, 2001).
3. Grant's zebra embryos transferred into domestic mares (*Summers et al.*, 1987).

Thus, Applicant believes that the pending claims are fully enabled and commensurate in scope with his contribution to the art, and respectfully requests that the rejection be withdrawn.

2.6 REJECTION OF CLAIMS UNDER 35 U. S. C. § 112, 2ND PARAGRAPH, IS OVERCOME.

At pages 12 to 14 of the present Action, claims 2, 9, 13, 17, 23, 29-32, AND 59-62 were rejected under 35 U. S. C. §112, 2nd paragraph, allegedly as being indefinite.

Applicant respectfully traverses.

However, in the interest of advancing certain claims of commercial importance to early allowance, Applicant has made the following amendments. Applicant notes that such amendments are in no way an indication of Applicant's acquiescence to the Examiner's rejection, and expressly reserve the right to re-file claims directed to the original scope of the amended claims.

Claims 2, 9, 13, and 17 have been amended to improve the clarity of the language of the claim, and to properly provide the required antecedent basis for the dependent claims.

As claims 21, 23, 29, 30, and 31 have been canceled, the rejection of these claims is now moot.

Applicant respectfully requests that the rejection now be withdrawn.

2.7 THE REJECTIONS OF CLAIMS UNDER 35 U. S. C. § 102(b) ARE RENDERED MOOT.

At page 14, the Action rejected claims 20, 22, 23, 28, 29, and 33 under 35 U. S. C. §102(b) allegedly as being anticipated by McLaughlin et al. (Reprod. Fertil. Dev., 2:619-622 [1990]).

Applicant respectfully traverses. However, in an interest of progressing certain aspects of the present case to allowance, mindful of patent term and economic issues of a protracted examination, and without acquiescing to the rejection in any way, Applicant has canceled the rejected claims. The rejection now being moot, Applicant respectfully requests that it be withdrawn.

At page 15, the Action rejected claims 20-23, 28-30, and 32-33 under 35 U. S. C. §102(b) allegedly as being anticipated by Schnieke et al. (Science, 278:2130-2133 [1997]).

Again, Applicant respectfully traverses; however, as indicated above, Applicant has canceled the rejected claims, and the rejection is now moot. Applicant respectfully requests that the rejection be withdrawn.

At page 16, the Action rejected claims 20-23, 28-33 under 35 U. S. C. §102(b) allegedly as being anticipated by Bowen et al. (Biol. Reprod., 50:664-668 [1994]).

Applicant again respectfully traverses the rejection; however, as indicated above, the rejected claims have been canceled, which renders the present rejection moot.

Applicant respectfully requests that this rejection under 35 U. S. C. §102(b) also now be withdrawn.

2.8 THE REJECTIONS OF CLAIMS UNDER 35 U. S. C. § 103(a) ARE OVERCOME.

At pages 16-20 of the Action, the Office rejected claims 1, 2, 5-13, 16-33, 56, 59-63 under 35 U. S. C. §103(a) allegedly as being unpatentable over Campbell et al. (PCT Intl. Appl. Publ. WO 97/07668 [6 March 1997]) in view of Boquest et al. (Biol. Reprod., 60:1013-1019 [1999]), and further in view of Alberts et al. (Mol. Biol. Cell, 3rd Ed. 1994, Garland Publishing, Inc. NY, pp. 903-904).

As to each rejection, the Applicant respectfully traverses.

Applicant notes for the record that a finding of obviousness under 35 U. S. C. § 103 requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the claimed subject matter and the prior art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. John Deere Co.*, 148 USPQ 459 (U.S. S.Ct. 1966).

The relevant inquiry is whether the prior art suggests the invention and whether the prior art would have provided one of ordinary skill in the art with a reasonable expectation of success.

In re O'Farrell, 7 USPQ 2d 1673 (Fed. Cir. 1988). Both the suggestion and the reasonable

expectation of success must be founded in the prior art and not in the Applicant's disclosure (emphasis added) *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

Furthermore, in the case of *In re Dow Chemical Co.* (837 F. 2d 469, 5, U.S.P.Q.2d 1529, Fed. Cir. 1988) the court held that an “obvious-to-experiment” standard is not an acceptable alternative for obviousness, and that there must be a reason or suggestion in the art, *other than* the knowledge learned from the Applicant’s disclosure.

Applicant also submits that the combination of references relied upon by the Examiner also clearly fails to satisfy the tripartite test of *In re O'Farrell* (7 U.S.P.Q.2d 1673, 1680, Fed. Cir. 1988). In *O'Farrell*, the Court held that in order for a single reference or a collection of references to obviate an invention, it must be shown that the reference(s) contain(s):

- (1) Detailed enabling methodology for practicing the claimed invention;
- (2) A suggestion for modifying the prior art to practice the claimed invention; and
- (3) Evidence suggesting that the invention would be successful.

For the cited combination of references to render the rejected and newly-presented claims legally obvious under 35 U. S. C. § 103, the references must provide one of ordinary skill in the art with a reasonable expectation of obtaining the results embodied by the pending claims.

Thus, Applicant respectfully disagrees with the Office’s contention that the subject matter of the present claims is legally obvious in view of the art of record. Applicant believes that the combination of references fails to meet the legal standard of *Vaeck* or *O'Farrell*, and as such the rejections must be withdrawn.

In particular, the Applicant disagrees that the present claims are legally obvious in view of *Campbell et al* when taken together with *Baquest et al* and *Alberts et al*; and over *Campbell et*

al in view of *Boquest et al*, *Prather et al*, *Gadbois et al* and *Collas et al.* and provides the following reasoning to support his position that the rejections are improper:

First, while *Campbell et al* (WO 97/07668) may claim methods of reconstituting an animal embryo by transferring a diploid nucleus in G0 or G1 phase into an enucleated oocyte, they certainly do not demonstrate the production of cloned embryos or animals with anything other than G0 donor cells. Indeed, prior literature using somatic cells for nuclear transfer had failed to generate cloned animals with any cells other than G0 donor cells. Thus, this reference is not enabled for G1 cells and together with the common general knowledge and prior art at the date of the invention, teaches away from the present invention.

The previous studies with G1 donors reported by *Collas et al* 1992 were limited to undifferentiated embryonic blastomeres and they did NOT use cultured differentiated (somatic) cells. It is only with the use of cultured differentiated cells that it is possible to clone an animal, as opposed to cloning an embryo. Whilst early studies with undifferentiated embryonic blastomeres did indeed demonstrate that cells in G1 would avoid chromosomal errors following nuclear transfer with non-activated oocyte cytoplasm, it had not been demonstrated that differentiated somatic cells in G1 could be reprogrammed following nuclear transfer and yield viable cloned offspring. At the time, it was not at all obvious that this was possible.

It must be remembered that before the birth of “Dolly” the sheep in 1996 (the first animal to be cloned from an adult) no one had produced a clone from a differentiated (somatic) cell.

Moreover, it was considered at the time that the major reason for that success was the use of differentiated donor cells that had been forced to exit the cell cycle and had entered a so-called quiescent, or G0, state (*Wilmut et al.*, 1997).

This view arose because from the pioneering cloning work in amphibians and in the mouse it was considered impossible to clone adult animals.

The criticality of the G0 donor cell state is reflected in a “News and Views” commentary, that was published in the same issue of *Nature* as the *Wilmut et al 1997* paper (*Stewart, 1997*). Colin Stewart states on line 3 of the legend for Figure 1 that “their success is largely due to the fact that nuclei were taken from cells in the G0 phase of the cell cycle”.

Wilmut et al 1997 also state on page 810, first paragraph, last sentence that “The birth of lambs from differentiated fetal and adult cells also reinforces previous speculation^{1,2} that by inducing donor cells to become quiescent it will be possible to obtain normal development from a wide variety of differentiated cells.” Additionally they state on page 812, column one, last paragraph, lines 5-6 that “Our studies with cultured cells suggest that there is an advantage if cells are quiescent (ref 1, and this work).” Hitherto, the production of viable cloned mammals from differentiated somatic cells had not been demonstrated.

The Examiner is further reminded that earlier publications report the failure to generate viable cloned offspring from differentiated cells using unsynchronized cell populations or populations in which a large proportion of the cells would have been expected to be in G1. For example, *Collas and Barnes* in 1994 had, prior to *Wilmut et al 1997*, used bovine granulosa cells, which possess a naturally long G1 phase, for nuclear transfer and failed to produce any cloned offspring from these somatic cells (*Collas and Barnes, 1994*). Similarly, unsynchronized sheep embryonic cells from late passage cultures, where a majority of the cells would be presumed to be in G1, also failed to result in cloned lambs (*Campbell et al., 1996*).

The fact that the first mammalian clones from differentiated cells were produced from G0 cells and previous attempts using G1 cells had failed, teaches away from believing that differentiated donor cells in G1 could be totipotent following nuclear transfer.

Given this, it was both surprising and unexpected from the studies reported in the present application that differentiated donor cells specifically selected in G1 of the donor cell cycle were indeed totipotent following nuclear transfer.

The present application also shows that with genetically modified fetal fibroblast donor cells, it was *even more surprising* that cloned embryos derived from G1 transgenic donor cells resulted in significantly greater rates of both development to term and the production of viable calves at weaning, compared to those cloned embryos derived from G0 donor cells (*Wells et al.*, 2003). The present application includes an initial part of the data reported in *Wells et al 2003* (e.g., see Fig 5).

The Examiner takes the position that *Boquest* and *Prather* provide a mechanism to segregate G0 from G1 cells in a mixed population. Applicant asserts, however, that this distinction is arbitrary and the method requires the fixation of cells which renders them non-viable and unable to be used successfully for nuclear transfer.

The Applicant asserts that neither *Boquest et al* nor *Prather et al* produces a pure population of cells at G1 stage of the cell cycle, nor any other cell cycle stage for that matter. They were only able to enrich for certain cell cycle stages but not select them for use in nuclear transfer. In addition, the methods used by *Boquest* and *Prather* produced cells which had been fixed in ethanol and therefore **cannot** be used in nuclear transfer. Thus, the method could not be used to select populations of G1 cells for nuclear transfer.

Taking the results disclosed in *Boquest* and *Prather* at face value, there was an enrichment of G1 cells to around 80%, by examining small cells from cycling cultures (Table 2 in *Prather* and Table 2 in *Boquest*). However, there are technique measurement factors that would make a person skilled in the art question these results.

The sorting methodology used *Boquest* and *Prather* relied on dual parameter flow cytometry, using simultaneous cellular DNA and protein content measures, in an effort to discriminate between different cell cycle phases within the cell population. The rationale is that as cells progress through the cell division cycle from G1, S, G2 and M phase, the DNA and protein content of the cell increases. G0 cells, that have exited the cell cycle, and G1 transient cells have the least protein and a 2C amount of DNA. However, there is no clear cut-off in protein or DNA content between cell cycle states as measured by flow cytometry. Especially in regard to protein content, it is a continuum (*e.g.*, see data in Figure 1, p1014 of *Boquest* and Fig 2 in *Prather*) and artificial boundaries are established to characterise cell cycle states.

Boquest et al (and it is similar for *Prather et al*) describes (Paragraph 2 of column 1, p1014) a visual method of discrimination of G0 from G1 by setting arbitrary gates (or boundaries) on a flow cytometer using a scatter-plot of green versus red fluorescence (See result in panel 1 of Figure 1). This combines protein and DNA measures, respectively. No biological corroboration is provided for the G0/G1 cut-off point chosen, and the fact that it was a subjective estimate is acknowledged on page 1015, column 2, paragraph 1. In fact, the chosen protein cut-off point for the G0/G1 boundary is set at a much lower level (at approximately 200 units; see Figure 1) than the cut-off level (at approximately 400 units) with the serum-starved 2C cells (see panel 2, protein vs. DNA in Figure 2), which is the accepted method of producing G0 cells.

In addition, natural variation in DNA and protein content of cells within a given cell cycle stage (Fig 1, and 2) combined with variation in the accuracy of measurement means that there will be significant error in this classification, given the continuum of data across the chosen boundary. The combination of all of these factors means that discrimination between different cell cycle stages in the sorting technique used must be considered arbitrary and will involve significant errors in the results obtained using this method. This is especially true in relation to setting the G0/G1 boundary. The Applicant, therefore, contends that the sub-population of cells claimed to be in G1 by the authors of *Boquest and Prather* in fact also contains G0 cells.

This sorting inaccuracy is material because of the low efficiency levels of cloning using nuclear transfer, and the inability to attribute cloning success to G0 or G1 donor cells in impure, mixed cell populations.

Thus *Boquest and Prather* does not teach an accurate and reliable method of isolating a pure population of cells. Furthermore, by following the teachings of *Boquest and Prather* the discrimination between G0 and G1 phases, which is critical to avoid infringement of other Intellectual Property, is not at all clear or defensible. And finally, the methods used by *Boquest and Prather* produces cells which have been fixed in ethanol and **cannot** be used in nuclear transfer to create a viable cloned embryo or animal.

Applicant respectfully requests, therefore, that the rejection be withdrawn.

At pages 20-23 of the Action, the Office rejected claims 1, 3, 4, and 64-66 under 35 U. S. C. §103(a) allegedly as being obvious in view of Campbell et al. Int'l. Appl. Publ. (WO 97/07668 [6 March 1997]) together with Boquest et al. (Biol. Reprod. 60:1013-1019 [1999]), and further in view of Prather et al. (Cloning, 1(1):17-24

[March 1999]), Gadbois et al.. (Proc. Natl. Acad. Sci. USA, 89:8626-8630 [September 1992]) and Collas et al. (Biol. Reprod., 46:492-500 [1992]).

Applicant again respectfully traverses.

While *Alberts* suggests senescent cells exit the cell cycle in G0, this is contrary to other studies which state that G0 (quiescence) and senescence are distinct and that senescent cells arrest in late G1 of the cell cycle (*Sherwood et al.*, 1998; *Pignolo et al.*, 1998). Although a cultured fibroblast cell line *might* contain a sub-population of senescent cells arrested in G1, it had not been previously demonstrated that such cells could be used for nuclear transfer to generate cloned fetuses (see example 7 of the present application).

The kinase inhibitors used by *Gadbois et al* 1992 enriched the G1 population to 91%, but these cells could not be used for nuclear transfer following selection on a fluorescent activated cell sorter, as the flow cytometry method used cells fixed in ethanol. Moreover, *Campbell et al* do not demonstrate that it is possible to clone an animal using G1 donor cells and indeed prior evidence taught away from this possibility. So, although methods *might* have been available prior to 1999 to arrest a high proportion of cells in G1 cells, they could not be selected by the methods of *Gadbois et al* for use in nuclear transfer and even if viable cells could be selected, it was not at all obvious at the time that G1 cells would result in successful somatic cell cloning.

Thus, Applicant does not consider that a skilled worker would be motivated to use an ethanol-fixed isolated cell of *Boquest/Prather/Godbois* in the method of nuclear transfer of *Campbell et al.* Such a method whether the cell was isolated in G0 or G1, would clearly **not** result in the production of a viable NT unit. Alternatively, if a skilled worker were to try and isolate and segregate a viable cell for NT, by following the teaching of *Cambell et al*, they would be motivated to try to isolate a cell in G0 (**NOT** G1).

Furthermore, Applicant has shown, for the first time, that somatic cells isolated in G1-phase of the cell cycle are totipotent following nuclear transfer, *i.e.*, G1 somatic cells can result in the birth of viable offspring. The present application describes for the first time a specific method that enables one to repeatedly isolate G1 donor cells at the time of nuclear transfer. This methodology was developed to ensure that each donor cell was indeed in a definitive G1 phase of the cell cycle. The important point is that the Applicant's methodology demonstrates that definitive G1 cells are totipotent. Therefore, it is a sound prediction that other methods that also enable the isolation of viable cells in G1 phase will be similarly useful for nuclear transfer.

In view of the combined literature cited by the examiner on this point, Applicant contends that it was not at all obvious at the date of the invention that a somatic cell in G1 could result in viable animals following NT. Prior work indicated that this was only possible with embryonic cells and not with somatic cells. The methods of flow cytometry did not allow the accurate segregation of an exclusive population of G1 donor cells that remained viable and useful for nuclear transfer.

The Applicant has also demonstrated that cloned animals produced by the method of the invention, *i.e.*, from segregated G1 donor cells, had surprisingly better survival rates in both gestational and post natal stages than for cloned animals produced from G0 cells. This is not what would have been expected from the prior art, which thus teaches away from the present invention.

Therefore, it is Applicant's position that the cited references (either alone, or in any combination) *cannot* obviate the claimed invention when the combination of references fails to provide both the required suggestion and the required reasonable expectation of success of generating the claimed invention.

Because the claims in the case particularly point out distinctly novel and non-obvious methods as disclosed in the Specification, and because each such claim is clearly distinguished over the cited art (either alone or in combination), Applicant believes that, as a matter of fact, the rejection advanced under 35 U. S. C. § 103 cannot stand. Applicant respectfully submits that all aspects of the instant 35 U. S. C. § 103 rejections have been overcome and withdrawal of the rejections is earnestly solicited.

2.9 CONCLUSION

It is respectfully submitted that all claims are fully enabled by the Specification, that all pending claims are enabled, definite, and free of the cited prior art, and that the inventions embodied in those claims are useful, novel, and non-obvious. Applicant believes that the claims are acceptable under all sections of the Statutes and are now in condition for ready allowance.

Applicant earnestly solicits concurrence by the Examiner and the issuance of a Notice of Allowance in the case with all due speed.

Applicant notes for the record his explicit right to re-file claims to one or more aspects of the invention as originally claimed in one or more continuing application(s) retaining the priority claim from the present and parent cases.

Should the Examiner have any questions, a telephone call to the undersigned Applicant's representative would be appreciated, and in particular in advance of any subsequent action on the merits.

Respectfully submitted,



Mark D. Moore, Ph.D.
Registration No. 42,903

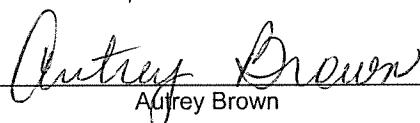
Date: October 13, 2006

HAYNES AND BOONE, LLP
901 Main Street, Suite 3100
Dallas, Texas 75202-3789
Telephone: 713-547-2040
Facsimile: 214 200-0853
36697.6

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Autrey Brown